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Public Health and Marine-Hospital Service of the United States.
WALTER WYMAN, Surgeon-General.

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M. J. ROSENAU, *Director*.
MAY, 1905.

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1905

CHLORIDE OF ZINC

Biological & Medical Serials

AS A

DEODORANT, ANTISEPTIC, AND GERMICIDE.

BY

T. B. McCLINTIC.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1905.

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No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

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[Continued on third page of cover.]

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CHLORIDE OF ZINC AS A DEODORANT, ANTISEPTIC, AND GERMICIDE.

By Thomas B. McClintic,
Passed Assistant Surgeon, U. S. Public Health and Marine-Hospital Service.

INTRODUCTION.

This compound of chlorine with zinc had a reputation as a deodorant and disinfectant for many years.

It was used for this purpose in a more or less empirical manner before we were aware of the existence of micro-organisms or the nature of putrefactive changes and long before it had been suspected that bacteriology would take the important rank in the field of science that it justly holds to-day. At the period when it seems to have had its greatest popularity in sanitary work the rationale of disinfection was very poorly understood. As the existence of germs was unknown at that time, the word "germicide" was not in use and the terms deodorant, antiseptic, and disinfectant were used more or less interchangeably and synonymously. Even to-day this is often the case. A substance may have practically no germicidal properties, but if it has the power to mask or destroy offensive odors arising from matter undergoing decay many persons will class it as a disinfectant.

This error is often productive of unfortunate consequences in handling and disposing of infected material and very much augments the difficulties that beset the sanitarian in the performance of his duties. It is too often observed, aboard ship, for instance, that the master considers his vessel disinfected after using a small quantity of one of the various preparations sold on the market as a disinfectant. The preparation used may or may not have germicidal properties; but, assuming the former condition to be the case, the quantity used is usually so limited that, as a rule, all that is accomplished is a certain amount of deodorization.

Of course there is, to a certain extent, a relation existing between the terms in question, a disinfectant usually being a deodorant, but a deodorant is by no means always a disinfectant or even an antiseptic.

Man naturally has an instinctive repugnance to all offensive exhalations, and from the earliest times has sought to overcome their presence in some way, and it is natural that any substance giving promise in this field should gain more or less popularity.

Not many years ago the inhalation of foul odors was regarded as one of the principal etiological factors concerned in the transmission of some of the infectious diseases, which may in part account for and have justified the use of chloride of zinc.

There is no doubt that the virtues accredited to chloride of zinc have been acquired largely through what power it may have for removing obnoxious effluvia and not because of its power to destroy microorganisms.

The literature covering the experimental work that has been done with chloride of zinc for the purpose of ascertaining its antiseptic and germicidal properties is meager, and the results that have been published by different investigators are not always satisfactory and sometimes contradictory.

REVIEW OF LITERATURE.

Chloride of zinc as a deodorant and disinfectant was very popular in the British navy[a] about the middle of the last century, some of the naval medical officers strongly recommending its use in the treatment of dysentery and in preventing the spread of cholera. It was found particularly useful in overcoming the disagreeable smells arising from the excretions of patients sick with these diseases.

The deputy inspector of royal naval hospitals in Bermuda published a report in 1854[b] in which he regarded chloride of zinc as a most powerful and instantaneous deodorant, and he, too, used it with great success in overcoming the foul emanations arising from patients lying in the wards in every stage of the worst forms of yellow fever. The waving of flags moistened with the solution and the employment of other means recommended for its use dissipated the foul air and restored freshness.

Concerning chloride of zinc as a disinfectant, the royal scientific deputation of medical officers,[c] 1856, states that there is no doubt and no further research is necessary to show that a solution of zinc chloride will perform very good service in protecting the bilge and preventing bilge water of ships from putrefying, and in improving the smell of water-closets, etc., but that it is not to be preferred to chloride of lime and chlorine in contagious diseases.

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[a] Royal Naval Medical Reports: Extracts from official reports upon the effects of chloride of zinc in deodorizing offensive effluvia from cesspools, sewers, etc., and in decomposing poisonous emanations from the bodies of those affected by contagious diseases. Med. Times and Gaz., London, 1853, n. s. 7, pp. 341–344.

[b] Hilditch, E.: Report on deodorizing and disinfecting properties of the chloride of zinc. Med. Times and Gaz., London, 1854, n. s. 9, p. 106.

[c] Ueber den Chlorzink als Desinfectionsmittel. Viertelj. f. gericht. und off. Medicin, Berlin, 1856, pp. 104–107.

In 1875 and 1876 Pettenkofer and Mehlhausen[a] directed a number of trials in the German fleet upon the disinfecting value of zinc chloride. Bilge water of a specific gravity 1017 to 1035, with a slightly alkaline reaction, at a temperature of 20° to 30° C., was treated with a solution of 50 to 60 per cent strength in the proportion of 1:100 of bilge. A grayish flocculent precipitate rapidly settled, leaving a nearly clear yellowish liquid. All odor ceased and the organisms seemed to be killed. At the end of four weeks the mixture showed no signs of change. One part of the solution to 1,000 of bilge caused a decrease in the odor; 2 to 1,000 completely removed sulphuretted hydrogen, much reduced the rancid smell, and preserved the liquid for fourteen days.

The German cholera commission of 1879[b] prescribed zinc chloride for the disinfection of bilge water.

Grace Calvert[a] found that a solution of albumin to which 1 per mille of zinc chloride was added required over forty days before germs developed.

Sternberg[c] in 1881 performed some experiments to ascertain the germicidal value of chloride of zinc. He inoculated three rabbits with 0.5, 1, and 2.5 per cent solutions, respectively, that had been mixed with the blood of a rabbit dead of mouse septicemia (*B. murisepticus*) and allowed the mixture to stand from 20 to 30 minutes before inoculation. The rabbit receiving the 1 per cent solution was the only one to die, the other two recovering.

Koch[d] in 1881 did some experimental work with zinc chloride on account of the reputation it had as a disinfectant. It was considered an efficient disinfectant in the proportion of one part in 1,000.

In his experiments he found that the *Micrococcus prodigiosus* was not injured in the least after two days' exposure in a percentage of 1:1,000. He did not observe any difference in the results of similar experiments with a percentage of 1:100 for the first 16 hours' exposure, but after this time observed that the power to develop when transplanted was somewhat diminished as compared with the controls. The *Micrococcus prodigiosus* was not entirely killed after an exposure of 48 hours in a percentage of 1:100.

The spores of anthrax and subtilis grew quite as vigorously after 48 hours' exposure in this percentage as if they had not been exposed.

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[a] Rideal: Disinfection and preservation of food. 1903, p. 152.

[b] Rideal: Disinfection and preservation of food. 1903, p. 153.

[c] Bull. Nat. Board Health U. S. A. 1881, v. 3, p. 21.

[d] Ueber disinfection. Mitt. kais. gesundh., 1881, p. 28.

The following table shows the results of his experiments with anthrax spores exposed in greater percentages of zinc chloride:

Percentage of zinc chloride.	Time of exposure in days.					
	1	3	5	10	20	30
5	+	+	+	+	+	+
2	+	+	+	+	+	+

The mark + indicates that the spores taken from the zinc chloride solution on the respective days had undiminished power to develop.

Anthrax spores exposed on silk threads in a percentage of 1:200 of zinc chloride dissolved in blood serum retained their power to develop after 48 hours' exposure equally as well as the controls. This he observed under the microscope.

From his experiments he concludes that $ZnCl_2$ in a percentage of 1:20 has no power to influence anthrax spores, so far as their development is concerned, after an exposure of one week.

He further concludes that it is a fact that zinc chloride has no marked power to prevent micro-organic growth, and that it is, indeed, incomprehensible to him that any considerable disinfecting value could be ascribed to this substance.

F. Bouillat[a] recognized zinc chloride as a good antiseptic, but found that a 5 per cent solution did not kill the spores of anthrax. To blood serum and egg albumen diluted with two to four times their volume of water he added zinc chloride and observed that it coagulated the albumen, forming an insoluble zinc albuminate, and that, provided enough of the salt be added to unite with the whole of the albumen, no growth can take place. In his experiments with this zinc albuminate he also observed that it has some power for inhibiting bacterial growth and the development of foul odors.

Miquel[b] placed chloride of zinc in his class 3 of substances as "strongly antiseptic" and states that 1.9 parts in 1,000 will prevent putrefaction in neutral beef bouillon. He places it in the same class with permanganate of potash, chloroform, cyanide of potash and carbolic acid. He also states that 1.9 parts of zinc chloride is equivalent to 3.2 parts of carbolic acid for preventing putrefactive changes in neutral beef bouillon.

Sternberg[c] says: "In the writer's experiments 1:200 destroyed *Micrococcus Pasteuri* in 2 hours, but a 2 per cent solution was required to kill pus cocci in the same time; spores of *Bacillus anthracis* were not destroyed by 2 hours' exposure in a 10 per cent solution, but a solution of 5 per cent killed the spores of *Bacillus subtilis* in the same time."

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[a] Beiträge zur lehre von der antisepsis, 1882.

[b] Les organismes vivants de l'atmosphere. 1883.

[c] Text-book of bacteriology, 1891, p. 195.

The disinfecting solution devised by Dr. C. B. Dudley[a] consists of a neutral solution of chlorides of copper and zinc and mercuric chloride put up in 8 ounce bottles and securely corked. Each bottle contains 2,400 grains of zinc chloride, 120 grains of cupric chloride, 10.5 grains of mercuric chloride, and 10 drops of an equal mixture of terbene and spirits of turpentine. According to Sedgwick,[b] who examined it for its efficiency, the chloride of copper is added to fix the sulphureted hydrogen and the zinc chloride on account of its deodorizing properties. Sedgwick found the disinfectant an efficient germicide, killing the spores of *B. subtilis* and *B. anthracis* in a few minutes, but did not observe that the zinc chloride materially contributes to its value as a disinfectant.

Sternberg[c] states that solutions of chloride of zinc are largely used in this country and in Europe for disinfecting purposes. It is an excellent antiseptic and deodorant, but its power to destroy disease germs has been very much overestimated. It may, however, be relied upon for the destruction of pathogenic organisms, in the absence of spores, in solutions which contain from 5 to 10 per cent of the salt.

On page 20 of the same essay he further states that "chloride of zinc in 10 per cent solution may be used to disinfect the dejecta of those sick with cholera or typhoid fever, or sulphate of copper in a solution of the same strength (10 per cent), the amount of the solution used being equal to the amount of material to be disinfected."

Under the name of "Burnett's disinfecting fluid"[d] chloride of zinc has had an extensive use in England. This fluid contains from 40 to 50 per cent of zinc chloride.

In Belgium infected clothes are boiled in a solution or a mixture of 240 grams of zinc sulphate and 120 grams of salt dissolved in a pail of water.[e]

The diversity of opinion and results of investigators concerning the value of chloride of zinc as a disinfectant is apparent, and it was for the purpose of determining the real merits of chloride of zinc as a deodorant, antiseptic, and germicide that this work was undertaken.

Acknowledgment.—To Dr. M. J. Rosenau, Director of the Hygienic Laboratory, I am gratefully indebted for assistance and suggestions in the performance of this work.

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[a] Pa. R. R. specifications for disinfectant No. 27.

[b] Technology quarterly, v. 6 (2), July, 1893.

[c] Sternberg: Lomb Prize Essay—Disinfection and individual prophylaxis against infectious diseases. p. 19. Revised edition, 1899.

[d] Rideal: Disinfection and preservation of foods, 1903.

[e] Rideal: Disinfection and preservation of food, 1903, p. 345.

PHYSICAL AND CHEMICAL PROPERTIES.

According to the United States Pharmacopœia, chloride of zinc ($ZnCl_2$), as found on the market, is a white, granular, friable, translucent powder or porcelain-like masses, irregular, or molded into pencils, odorless, and of such intensely caustic properties as to make tasting dangerous unless the salt be diluted, when it has an astringent, metallic taste.

It is very deliquescent, and owing to its hygroscopic character should be kept in bottles closed with paraffin. It is possessed of dehydrating powers, removing oxygen and hydrogen from organic matter in the form of water, which probably accounts for what influence it exerts as an antiseptic and germicide. It is soluble in about 0.3 part of water at 15° C., forming a clear, viscid solution, which on boiling deposits a basic salt.

It is practically impossible to obtain zinc chloride entirely free from basic salt, and the U. S. Pharmacopœia prescribes the limit of this by directing that 1 drop of hydrochloric acid shall clear up opacity caused in 5 c. c. of a 5 per cent aqueous solution of the salt by the addition of an equal volume of alcohol.

In somewhat aqueous solutions zinc chloride undergoes partial hydrolysis, the precipitate consisting of basic or hydroxy-chlorides, e. g., $ZnCl_2 + H_2O = HCl + Zn\left|\begin{matrix}HO\\Cl\end{matrix}\right.$. This flocculent precipitate can be cleared by the careful addition of dilute hydrochloric acid.

The U. S. Pharmacopœia requires that the official salt contain not less than 99.84 per cent of zinc chloride. This can be determined by dissolving 0.3 gram of dry chloride of zinc in 10 c. c. of water and adding 2 drops of chromate of potash, when it should require 44.1 c. c. of a decinormal silver solution to produce a permanent red color.

When heated to 115° C., zinc chloride fuses to a clear liquid. At a higher temperature it is partly volatilized in dense, white fumes and, in part, decomposed, leaving a residue of zinc oxide.

The aqueous solution turns blue litmus paper red.

A 5 per cent solution produces no corrosive action on iron, brass, wood, or caoutchouc, and, it is said, does not rot ordinary fabrics, but causes a reddening and smarting sensation when applied to the skin.

Linen threads and hair are not apparently affected after thirty days exposure to a 100 per cent solution of zinc chlorid, but the same strength solution gradually destroys cotton and silk threads. This destruction is so complete at the end of ten or eleven days that no evidence of the presence of these materials in the solution is observable.

The Pharmacopœial solution of zinc chloride (liquor zinci chloridi) has a specific gravity of about 1.535 at 15° C. and contains about 50 per cent by weight of the salt.

It is practically identical with Burnett's disinfecting fluid.

In the preparation of the percentages of zinc chloride used in the following work 100 per cent solution was first prepared by adding enough distilled water to a given number of grams of the dry zinc chloride to make the same number of cubic centimeters of solution, e. g., to 100 grams of dry zinc chloride sufficient water was added to make 100 c. c. of solution. This solution was kept as a stock from which the weaker dilutions were made.

PROPERTIES AS A DEODORANT.

Chloride of zinc is capable of combining with hydrogen sulphide, ammonia, and other offensive products of putrefaction, forming compounds that are comparatively odorless; hence, zinc chloride is a deodorant. It is probable that this property is more or less responsible for its historical reputation as a disinfectant.

Zinc chloride unites with the ammonia given off from organic matter undergoing decay to form ammonium chloride, zinc hydroxide, and various other compounds. With hydrogen sulphide it unites to form zinc sulphide, but the union is so feeble that it is rather easily broken up again into hydrogen sulphide, etc. As the sulphide of copper is a more stable compound than the sulphide of zinc, chloride of copper is sometimes used for the purpose of fixing the hydrogen sulphide.

Experiments were performed to ascertain the value of chloride of zinc as a deodorant. Its properties as a deodorant were also observed and noted in the experiments that were conducted to determine its antiseptic powers.

Erlenmeyer flasks were partly filled with definite quantities of sewage (human excreta, stable manure, etc.) and varying definite quantities of zinc chloride added in order to determine its deodorizing powers. As this property is a more or less variable one depending upon time, temperature, and kind and quantity of organic matter present, one series was kept at room temperature and another placed in the incubating room at a temperature of 37° C. The zinc chloride was added in strengths varying from 1:10,000 to 1:50. It was observed that when zinc chloride was added to the above solutions in a proportion as little as 1 part in 10,000 a slight diminution of the odor was at once perceptible. This became more marked as the proportion of zinc chloride was increased until in the strength of 1:300 only slight odor could be detected. In a strength of 1:200 the odor was practically destroyed.

In the series allowed to stand at incubator temperature the deodorizing power gradually diminished. At the end of ten days a slight, but not disagreeable, odor was present in dilutions of 1:50, increasing in the higher dilutions until in strengths of 1:300 the odor became offensive. In the flasks kept at room temperature the changes were similar to but not so marked as in the incubator series.

Zinc chloride is still less efficient as a deordant in the presence of organic matter of vegetable nature. Some flasks were prepared as above with the addition of particles of vegetable matter, such as cabbage, etc., and kept at room temperature. In the flasks containing dilutions of 1:300 the odor gradually increased as the vegetable matter underwent decay until about the seventh day, when the odor was very offensive. No odor was appreciable in strengths of 1:50.

In dilutions of 1:200 the vegetable matter was preserved at the end of 14 days and the odor was very mild in comparison with the odor from the flasks containing the dilutions of 1:300. In those flasks that contained a less percentage of zinc chloride than 1:200 the degree of odor and the time necessary for decay and disappearance of the vegetable matter varied inversely as the proportion of zinc chloride added.

The deodorizing value of zinc chloride was next determined in the presence of less organic matter than the above experiments. For this purpose ordinary bouillon was inoculated with garden earth containing various organisms and allowed to stand at room temperature, under which conditions chloride of zinc will inhibit the production of foul odors in strengths of about 1:500.

ANTISEPTIC PROPERTIES.

For this purpose Erlenmeyer flasks of about 100 c. c. capacity were partly filled with nutrient bouillon and the zinc chloride added in various definite percentages.

When prepared in this way the chloride of zinc produces a white flocculent precipitate, which, on standing, settles to the bottom, leaving the upper portion of the liquid its original straw color and making it possible to observe any bacterial growth that may take place. The quantity of this precipitate varies directly as the quantity of chloride of zinc added.

The flasks having been prepared in this way were abundantly inoculated with different materials rich with organisms such as wisps of hay, fresh stable manure, garden earth, etc., and placed at room temperature. From day to day, up to the end of the fourteenth day, the appearance of molds and bacterial growths was observed. In case of doubt as to bacterial growths, hanging drop preparations were examined under the microscope.

Some of the results may be observed in the following tables:

Table A.—*Restraining power of zinc chloride prepared in bouillon and contaminated with wisps of hay—Kept at room temperature.*

[+ means bacterial growth; – means no bacterial growth; s. m. means surface mold; b. m., mold on the bottom; 0 means no growth of any kind.

Percentage used.	Day on which growth appeared.							
	Second.	Third.	Sixth.	Seventh.	Tenth.	Eleventh.	Twelfth.	Fourteenth.
1:1000	s. m. –	s. m. +	....	....	....	....	....	....
1:500	s. m. –	s. m. +	....	....	....	....	....	....
1:300	0	s. m. –	s. m. +	....	....	....	....	....
1:200	0	0	0	s. m. –	....	s. m. +	....	....
1:100	0	0	0	0	s. m. –	....	....	s. m. –
1:75	0	0	0	0	s. m. –	....	....	s. m. –
1:50	0	0	0	0	0	0	[a] Mold.	[a] Mold.
1:45	0	0	0	0	0	0	0	0
1:40	0	0	0	0	0	0	0	0
1:35	0	0	0	0	0	0	0	0

[a] This was only mold on head of hay and was really not in the solution.

Table B.—*Restraining power of zinc chloride prepared in bouillon and inoculated with garden earth—Kept at room temperature.*

Percentage used.	Day on which growth appeared.								
	Second.	Third.	Fourth.	Fifth.	Sixth.	Seventh.	Tenth.	Thirteenth.	Fourteenth.
1:1000	s. m. +	....	....	....	....	....	....	....	....
1:500	s. m. +	....	....	....	....	....	....	....	....
1:300	0	+	s. m. +	....	....	....	....	....	....
1:200	0	0	0	s. m. –	s. m. –	....	....	....	....
1:100	0	0	0	s. m.	....	....	....	....	s. m. –
1:75	0	0	0	0	0	b. m.	....	....	b. m. –
1:50	0	0	0	0	0	0	s. m.	....	s. m.
1:45	0	0	0	0	0	0	s. m.	....	s. m.
1:40	0	0	0	0	0	0	0	0	b. m. –
1:35	0	0	0	0	0	0	0	0	0

Table C.—*Restraining power of zinc chloride prepared in bouillon and contaminated with stable manure—Kept at room temperature.*

Percentage used.	Day on which growth appeared.								
	Second.	Third.	Fourth.	Fifth.	Eighth.	Tenth.	Eleventh.	Twelfth.	Fourteenth.
1:1000	s. m. +	....	....	....	....	....	....	....	....
1:500	0	s. m. +	....	....	....	....	....	....	....
1:300	0		s. m. +	....	....	....	....	....	....
1:200	0	0	0	s. m.	s. m. +	....	....	....	....
1:100	0	0	0	0	0	b. m. –	....	....	b. m. –
1:75	0	0	0	0	0	0	b. m.	....	b. m. –
1:50	0	0	0	0	0	0	0	b. m.	b. m. –
1:45	0	0	0	0	0	0	0	b. m. –	b. m. –
1:40	0	0	0	0	0	0	0	b. m.	b. m.
1:35	0	0	0	0	0	0	0	0	0

These experiments were repeated and the results verified.

In these experiments it will be observed that the minimum strength of chloride of zinc that inhibited the growth of molds for 14 days was about 1 part in 40, varying slightly with the character of the material used for contamination. The strength that prevented bacterial growth in the above experiments was between 1:200 and 1:100, while further and similar experiments showed that a percentage of 1:125 inhibits bacterial growth for 21 days, but a growth occurred on the fourteenth day in a percentage of 1:150.

It must be remembered that the antiseptic property of a substance varies more or less with light, character of medium used, and the temperature, the relative importance of these factors increasing in the order named. The influence of diffused light is comparatively feeble. The condition of the medium, independent of the presence of the substance whose antiseptic value is being determined, is an influential factor. A strongly alkaline or acid medium is usually inimical to the growth of bacteria, and the nutrition may not be favorable for the development of the micro-organisms present. A low temperature will inhibit bacterial growth and a high one destroy bacterial life.

Experiments were performed with sewage to determine the quantity of zinc chloride necessary to prevent the development of bacteria. A series of flasks was prepared with an average sample of sewage. Zinc chloride varying in proportion from 1:500 to 1:40 was added to this series of flasks, which were placed at room temperature. A slight cloudiness appeared on the tenth day in the flasks of sewage containing a percentage of 1:500 of zinc chloride.

Another series of flasks was prepared by mixing equal quantities by volume of sewage and tap water. These were also kept at room temperature, and it was found under these conditions that in the percentage of 1:500 of zinc chloride a bacterial growth developed on the fourteenth day.

In a third series of flasks containing sewage and nutrient bouillon in equal volumes and kept at room temperature as above it was found that a bacterial growth developed, indicated by the clouding of the bouillon, on the fifth day in the flask containing zinc chloride in the strength of 1:200, but not in a strength of 1:100 in 14 days.

The organic matter in the flasks referred to above was soon precipitated after adding the zinc chloride, and this was particularly the case when the zinc chloride was in quantity approximately sufficient to inhibit bacterial growth. This precipitate, or residue, was at first brownish in color, but soon became more or less bleached. The bleaching was more apparent in those flasks receiving sufficient zinc chloride to inhibit bacterial development, but was observed to a certain extent in all the flasks, the degree of which varied directly with the quantity of zinc chloride present.

After the precipitate in these flasks settled to the bottom the supernatant liquid was practically clear and transparent, and when the zinc chloride was in quantity sufficient to prevent micro-organic development it gradually assumed a straw color resembling nutrient bouillon.

Further work was done with these same flasks in order to determine the condition of this supernatant liquid and residue as regards sterility. In order to ascertain this, inoculations were made from the supernatant portion af each flask by transferring with a sterile pipette 4 or 5 drops of it to nutrient bouillon and placing it in the incubator at 37° C. Considering the absence of growth under these conditions as evidence of sterility, the following tables will show the results obtained with the supernatant portion of the different series of flasks containing various percentages of zinc chloride:

TABLE A.—*Germicidal influence upon the supernatant liquid when zinc chloride is added to ordinary sewage and kept at room temperature.*

[+ means growth; — means no growth.]

Percentage $ZnCl_2$.	Time of exposure in days.					
	6.	7.	9.	10.	12.	14.
1:500	—	+	+	+	+	+
1:200	—	+	+	+	+	+
1:100	—	+	+	+	+	—
1:75		+	+	+	—	
1:50	+	+	—	—	—	—
1:45	+	+	—	—	—	
1:40	+	—	—	—	—	—

TABLE B.—*Germicidal influence upon the supernatant liquid when zinc chloride is added to equal volumes of ordinary sewage and tap water and kept at room temperature.*

Percentage $ZnCl_2$.	Time of exposure in days.					
	6.	7.	9.	10.	12.	14.
1:500	—	—	—	—	—	—
1:200	—	—	—	—	—	—
1:100	—	—	—	—	—	
1:75	—	—	—	—	—	
1:50	—	—	—	—	—	—
1:45	—	—	—	—	—	
1:40	—	—	—	—	—	

TABLE C.—*Germicidal influence upon the supernatant liquid when zinc chloride is added to ordinary sewage and bouillon and kept at room temperature.*

Percentage $ZnCl_2$.	Time of exposure in days.			
	6.	7.	10.	14.
1:500	+	+	—	—
1:200	+	+	—	—
1:100	+	+	—	—
1:75	+	+	—	—
1:50	+	+	—	—
1:45	+	+	—	—
1:40	+	—	—	—

From the above results it will be observed, as might be expected, that the microorganisms remained viable longer in the flasks containing the most nutrition, other things being equal. The supernatant portions of the two series of flasks containing no nutrient bouillon were sterile at the end of 14 days in a proportion of zinc chloride of

1:100, while in the series to which bouillon was added it was sterile at the end of the same time (14 days) only in a proportion of 1:75.

Inoculations from the residue in the bottoms of these same flasks made into nutrient bouillon in the same way as the above showed that some microorganisms, probably spore-bearing, were still viable and capable of multiplying in all the flasks after standing 21 days. As can be observed, the largest percentages of zinc chloride used in these series of experiments were 1:40.

A series of flasks was next prepared containing ordinary sewage, to which was added particles of vegetable matter, such as cabbage, etc. Zinc chloride was added in definite quantities varying from 1:1,000 to 1:40. The flasks were kept at room temperature. At the end of 14 days the particles of floating cabbage were still well preserved and the supernatant liquid was a straw color in those flasks containing zinc chloride in a proportion as large as 1:200.

In the flasks of this series containing zinc chloride in the proportion as small as 1:500 the liquid took on a brownish cloudy appearance and the particles of cabbage gradually disappeared. This decay and disappearance of the vegetable matter was of course more rapid in the higher dilutions of zinc chloride.

In the foregoing experiments for ascertaining the antiseptic properties of zinc chloride the solutions always became decidedly acid, which has a certain tendency to inhibit decay and the growth of microorganisms.

GERMICIDAL PROPERTIES.

For determining the germicidal value of chloride of zinc various definite percentages were prepared in distilled water and about 4.5 c. c. of each strength placed in a test tube. These were then inoculated by adding 0.5 c. c. of an emulsion, in distilled water, of the organisms to be used, carrying over as little organic matter as possible.

Cultures of the different organisms used for this purpose were grown on agar slants for 24 hours at a temperature of 37° C.

Plants were then made in sterile bouillon at definite intervals by means of a wire loop. They were then placed in the incubator at a temperature of 37° C. and the results noted from day to day.

The results of the germicidal action of zinc chloride upon pure cultures of the different organisms used are shown in the following tables:

[+ = growth; − = no growth.]

BACILLUS COLI COMMUNIS.

Percentages used	Time of exposure in minutes										
	1	3	5	10	15	20	25	30	40	50	60
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

BACILLUS TYPHOSUS.

Percentage used.	Time of exposure in minutes.									
	1.	2.	5.	10.	15.	20.	30.	40.	50.	60.
5	+	+	+	+	+	+	+	+	+	+
10	+	+	+	+	—	—	—	—	—	—
15	+	+	—	—	—	—		—	—	—
25	+	—	—	—		—	—	—	—	—

VIBRIO CHOLERÆ.

Percentage used.	Time of exposure in minutes.										
	1.	2.	5.	7.	10.	15.	20.	30.	40.	50.	60.
0.5	+	—	—	+	+	+	+	+	—	—	—
1.0	+	+	—	+	+	+	—	—	—	—	—
3.0	+	+	+	+	+	—	—	—	—		
5.0	+	+	+		—		—	—	—	—	
10.0	+				—		—	—	—	—	—

BACILLUS DYSENTERIÆ (SHIGA).

Percentage used.	Time of exposure in minutes.							
	1.	4.	5.	10.	20.	30.	50.	60.
5	+	+	—	—	+	+	+	+
10	+	+	+	—	+	+	—	—
15	+	—	—	+	—	—		
25	+	+	—	—	—	—	—	—

BACILLUS DIPHTHERIÆ.

Percentage used.	Time of exposure in minutes.							
	1.	3.	5.	10.	20.	30.	50.	60.
3	+	—	+	+	+	+	+	+
5	+	—	+	+				—
10	+	—	—	—	—	—	—	—
25	+	—	—	—			—	—

STAPHYLOCOCCUS PYOGENES AUREUS.

Percentage used.	Time of exposure in minutes.							
	5.	10.	15.	20.	30.	40.	50.	60.
5	+	[illegible]	+	+	+	+	+	+
10	+	+	+	+	+	+	+	—
15	+	+	+	+	—	—	—	—
25	+	+	+	—	—	—	—	—

STAPHYLOCOCCUS EPIDERMIS ALBUS.

Percentage used.	Time of exposure in minutes.								
	1.	3.	5.	10.	20.	30.	40.	50.	60.
3	+	+	+	+	—	+	—	+	—
5	+	+	+	—	—	—	+	—	—
10	+	+	+	—	—	—	—	—	—
15	+	+	+	+	—	—	—	—	—
25	+	+	—	—	—	—	—	—	—

Controls all grew within 24 hours.

The foregoing results demonstrate that under the most favorable conditions zinc chloride is inefficient as a germicide for practical purposes, as it will be observed that the pyogenic organism *Staphylococcus pyogenes aureus* lives for 50 minutes in a strength of 10 per cent and 20 minutes in a strength of 25 per cent.

Bacillus coli communis was viable after exposure for 20 minutes in a 10 per cent solution and after 8 minutes in a 25 per cent solution.

The *Vibrio choleræ* was killed in a 5 per cent solution within 7 minutes and within 2 minutes in a 10 per cent strength. This is the only organism that was destroyed by a reasonable strength in a reasonable time.

In the experiments for determining the germicidal action of zinc chloride on the *Bacillus coli communis* it was desired to ascertain whether the organisms were really dead in the time and strengths that apparently were germicidal or simply overcome and still capable of reviving in case the zinc chloride was immediately removed or neutralized. Consequently some of the organisms were treated with ammonium sulphide for a few seconds, washed in distilled water, and planted in bouillon. It was not observed, however, that this appreciably altered the time required for germicidal action, indicating that the organisms were really killed.

To ascertain the germicidal value of zinc chloride in the presence of organic matter as compared with its value in distilled water experiments were performed with this end in view.

Equal percentages of zinc chloride were prepared in distilled water and nutrient bouillon, respectively, and inoculated with different organisms, after which the technique was the same as above described. The results are shown in the following tables, where comparisons may be made.

TABLE X.—*Germicidal results when the [illegible] was exposed in percentages of zinc chloride prepared in distilled water.*

Percentage used.	Time of exposure.								
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] hours.	[illegible] hours.
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

TABLE XI.—*Same as Table X, when nutrient bouillon was used instead of distilled water.*

Percentage used.	Time of exposure.									
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2 [illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table B.—*Germicidal results when the B. pyocyaneus was exposed in percentages of zinc chloride prepared in distilled water.*

Percentage $ZnCl_2$.	Time of exposure.							
	10 minutes.	20 minutes.	30 minutes.	2 hours.	3 hours.	5 hours.	7 hours.	18 hours.
5	+	+	−	+	+	−	+	−
10		+	+	+	−	−		
25	−	+		−	−	−	−	−

Table B1.—*Same as Table B, when nutrient bouillon was used instead of distilled water.*

Percentage $ZnCl_2$.	Time of exposure.							
	10 minutes.	20 minutes.	30 minutes.	2 hours.	3 hours.	5 hours.	7 hours.	18 hours.
5	+	+	+	+	+	+	+	+
10	−	+	+	+	−	+	−	−
25	+	+		−	−	−	−	−

These results, confirmed by repeating several times, show that when zinc chloride is prepared in nutrient bouillon its germicidal value is not appreciably influenced by the organic matter contained therein as compared with its value when prepared in distilled water.

As has already been stated, zinc chloride produces a heavy white precipitate when added to nutrient bouillon. Consequently experiments were performed to determine the relative germicidal properties of this precipitate as compared with those of the clear transparent portion that remains above. A 10 per cent solution of zinc chloride in nutrient bouillon was prepared and allowed to settle. The clear supernatant portion was then siphoned off and 10 c. c. of it placed in a test tube. Ten cubic centimeters of the precipitate was placed in another test tube. Both tubes were then inoculated with a 24-hours-old agar culture of *Bacillus pyocyaneus*, after which plants at definite intervals were made from each tube into nutrient bouillon in the usual way.

The results of the inoculations made every hour show that the *Bacillus pyocyaneus* was killed within 3 hours in the tube containing the supernatant portion, and within 4 hours in the tube containing the precipitate, indicating that the supernatant portion has slightly greater germicidal powers than the precipitated portion. A similar experiment in which *Bacillus typhosus* was substituted for *Bacillus pyocyaneus* shows that this organism was killed in both tubes within 2 hours.

GERMICIDAL INFLUENCE OF ZINC CHLORIDE ON SEWAGE.

For this work an average sample of sewage was selected. Definite quantities were placed in Erlenmeyer flasks and heavily inoculated with a watery suspension of a 24 hours-old agar culture of *Bacillus pyocyaneus*. Various definite percentages of zinc chloride were then added and the flasks kept at room temperature. At definite intervals inoculations were made into bouillon and Dunham's solution in the usual manner and placed in the incubator at 37° C.

In the following results P means that there was a growth of *pyocyaneus;* + means that there was a bacterial growth, but no evidence of *pyocyaneus;* – means no growth.

Percentage Zinc.	Time of exposure.										
	1 hour.	2 hours	3 hours	5 hours	1 day.	2 days.	3 days.	4 days.	5 days.	7 days.	14 days.
[illegible]	P	P	P	P	P	P	P	P	P	P	+
[illegible]	P	P	P	P	P	P	P	P	P	P	+
[illegible]	P	P	P	P	P	P	P	P	P	-	+
[illegible]	P	P	P	P	-	-	-		+	-	+
[illegible]	P	P	—	-	-	-	-	—	+	+	+
[illegible]	—	+	-	-	-	-	-		+		-

Bacillus pyocyaneus was used in these experiments as a control, so to speak, on account of the ease with which its growth can be observed.

As its power of resisting the action of germicides compares very favorably with that of the nonspore-bearing pathogenic organisms it possesses value for control purposes in the disinfection of feces. When exposed to the action of zinc chloride prepared in sewage—with its host of various micro organisms—the viability of the *B. pyocyaneus* is diminished as compared with its viability when exposed in the same strengths of zinc chloride prepared in distilled water. This difference is rather marked and may be due to a combination of factors.

The sewage was slightly acid and of course contained many other organisms which in time may have overrun the pyocyaneus, as it had disappeared from the control flask at the end of 14 days.

By referring to the preceding tables it will be observed that *B. pyocyaneus* was killed within 3 hours in the flask of sewage containing 5 per cent of zinc chloride, while it was not destroyed after 7 hours' exposure in an aqueous solution of the same strength.

The organisms that were still viable after 1 or 2 days' exposure in the flasks of sewage containing the larger percentages of zinc chloride appeared to be principally spore-bearing organisms.

It was next desired to determine the viability of some of the pathogenic organisms, such as cholera, typhoid fever, etc., when exposed to the action of zinc chloride in sewage.

As the overgrowth of ordinary sewage bacteria makes this determination in sewage well nigh impossible, a sample of sewage similar to that used in the above experiments was sterilized with steam under pressure. After sterilization it was placed in flasks as in the foregoing experiment and heavily inoculated with 24 hours-old agar cultures of different pathogenic organisms and the various percentages of zinc chloride added.

Inoculations into bouillon at definite intervals were made and placed in the incubator at 37° C. Following are the results with the different organisms used:

Results when B. dysenteria (Shiga) was exposed in percentages of zinc chloride prepared in sterile sewage.

[+ means growth; — means no growth.]

Percentage $ZnCl_2$	Time of exposure in hours.					
	1.	2.	3.	5.	7.	24.
1	+	+	+	+	+	+
2	+	+	+	+	+	—
5	+	+	+	+	+	—

Results when B. typhosus was exposed in percentages of zinc chloride prepared in sterile sewage.

Percentage $ZnCl_2$.	Exposure in hours.				
	½.	1.	2.	3.	5.
1	+	+	+	+	—
2	+	+	—	—	—
5	+	—	—	—	—

Results when Vibrio choleræ was exposed in percentages of zinc chloride prepared in sterile sewage.

Percentage $ZnCl_2$.	Exposure in hours.				
	½.	1.	2.	3.	5.
1	+	+	+	+	—
2	+	—	—	—	—
5	—	—	—	—	—

Results when B. pyocyaneus was exposed in percentages of zinc chloride prepared in sterile sewage.

Percentage $ZnCl_2$.	Exposure in hours.				
	1.	2.	3.	5.	72.
1	+	+	+	+	+
2	+	+	+	+	+
5	+	[illegible]	+		

Controls were all viable at the end of 3 days

Sterilization of sewage of course alters its natural composition to a certain extent, but it is the only practical way in which the viability of the pathogenic organisms used in these experiments with zinc chloride in sewage can be determined with any degree of accuracy.

A comparison of the results obtained under these conditions show that they do not differ materially from those obtained under similar conditions when distilled water is used instead of sterile sewage.

EFFECTS OF ZINC CHLORIDE UPON SPORE-BEARING ORGANISMS.

Experiments were performed with 7-days-old agar cultures of *B. subtilis* and *B. anthracis*. A hanging-drop preparation of each culture was examined before it was used to see that it contained many spores.

Preparations of zinc chloride varying from 5 to 100 per cent were prepared in distilled water and 20 c. c. of each strength placed in a large test tube. It may be well to state here that by a 100 per cent solution of zinc chloride is meant a solution made by adding enough distilled water to a given number of grams of dry zinc chloride to make the same number of cubic centimeters of solution. The dilutions were made accordingly. For the purpose of exposing the spores in these solutions of zinc chloride different methods were employed. In some experiments a watery suspension of the organisms to be used was made and added to the different solutions of zinc chloride, after which plants from each tube into nutrient bouillon were made at definite intervals and placed in the incubator at 37° C.

The results show that the spores of *B. subtilis* were still viable after 4 days' exposure in a 100 per cent solution of zinc chloride, but produced no growth when transplanted after 5 days' exposure in the same strength. The spores of *B. anthracis* were capable of multiplying when transplanted into bouillon after an exposure of 7 days in a 25 per cent solution of zinc chloride.

An objection to the above mode of procedure is that when the inoculated solutions of zinc chloride are to be kept for a long period, say a month, some of the organisms that adhere to the sides of the test tube above the liquid, and consequently are not immersed in it, are liable to be carried over in making the plants from time to time and give erroneous results as to the germicidal value of the substance being determined or the viability of the organism under test.

This is more likely to occur if the contents of the tube are shaken up before making the inoculations.

By using a sterile pipette for making the inoculations and carefully carrying it to the bottom of the tube, as was done in the above experiments, this possible source of error is reduced to a minimum. The principal objection to the use of the pipette for this purpose is that too much of the zinc-chloride solution is carried over.

The silk thread method was also used for exposing the organisms in the zinc-chloride solutions and proved more satisfactory than the preceding method. Sterile silk threads about 1 inch long were soaked in a thick watery suspension of the organism to be used and about 20 threads placed in each strength of zinc chloride. Inoculations were made at definite intervals by carefully transferring a thread to nutrient bouillon and placing in the incubator at 37° C.

In the more concentrated solutions so much of the zinc chloride is carried over on the thread that it was found better to use two tubes of bouillon for each thread, one being used to wash out most of the zinc chloride, after which the thread is planted in another tube. Both tubes were then incubated at 37° C. The results obtained with the tubes in which the threads were placed after washing were much more uniform and satisfactory than those in which the threads were washed.

The following are the tabulated results with 7 days old spores of *B. anthracis* on silk threads exposed in various percentages of zinc chloride prepared in distilled water:

[+ means growth; –, no growth.]

Percentage $ZnCl_2$.	Time of exposure in days.					
	5.	10.	15.	20.	25.	40.
5	+	+	+	+	+	+
10	+	+	+	+	+	+
25	+	+	+	+	+	+
50	+	+	+	+	+	+

The spores of *B. subtilis* on silk threads exposed in a 100 per cent solution of zinc chloride were viable at the end of the tenth day, after which time the silk threads were so completely destroyed by the action of the zinc chloride that further planting was impossible.

By substituting linen threads, which are not destroyed by a 100 per cent solution of zinc chloride, for silk threads in the above experiment it was found that the spores of *B. subtilis* were still viable after thirty days exposure.

SUMMARY AND CONCLUSIONS.

Chloride of zinc has had more or less reputation as a deodorant, antiseptic, and disinfectant for many years, but it has gradually been realized that it was much overrated, particularly as an antiseptic and disinfectant, and its use for these purposes is becoming more and more limited.

It is used as an ingredient of some proprietary preparations found on the market and vaunted for their disinfectant powers, but its rôle is principally that of a deodorant. Even as a deodorant there are certain objections or limitations to its use, but for the sanitarian it is the only property of any practical value that it possesses.

When zinc chloride is added to sewage in dilutions as high as 1:10,000 a diminution of offensive odors is appreciable; but this effect is only transitory. As the percentage of zinc chloride is increased its deodorizing effect becomes more marked and more permanent. It is a fairly reliable deodorant in proportions varying from 1:500 to 1:200, but the exact percentage to be used in each case depends upon the kind and condition of material to be acted upon.

The union of zinc chloride with hydrogen sulphide is not stable enough to recommend it for the neutralization of this offensive compound.

The antiseptic powers of zinc chloride are feeble.

Prepared in nutrient bouillon a percentage of about 1:40 is required to inhibit the growth of molds for 14 days, while under the same conditions a strength of 1:125 is required to inhibit for the same length of time bacterial development.

Added to ordinary sewage, zinc chloride will inhibit bacterial growths in proportions varying from 1:500 to 1:200, depending to a certain extent upon the quantity of nutrition present.

Chloride of zinc can not be relied upon for destroying micro-organic life, as the *B. coli communis* is not killed in a 5 per cent solution in 1 hour's exposure, and it takes 10 minutes for a 25 per cent solution to kill the same organism.

It requires 30 minutes for a 25 per cent solution to kill *Staphylococcus pyogenes aureus*.

Spores of *B. subtilis* are not killed in a 100 per cent solution in 30 days, and the spores of *B. anthracis* are not killed in a 50 per cent solution in 40 days.

Zinc chloride has some properties as a deodorant to recommend it favorably, but its antiseptic and germicidal powers are comparatively feeble, which, with its cost and caustic properties, practically eliminate it from the useful and reliable disinfectants.

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No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

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